

## ORIGINAL ARTICLE

# Single nucleotide polymorphism in *RECQL* and survival in resectable pancreatic adenocarcinoma

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## Abstract

**Background:** *RECQL* is a DNA helicase involved in DNA mismatch repair. The *RECQL* polymorphism, 3' untranslated region (UTR) A159C, was previously associated with overall survival of patients with resectable pancreatic adenocarcinoma treated with neoadjuvant chemoradiation. In the present study, we examined *RECQL* for somatic mutations and other polymorphisms and compared these findings with the outcome in patients who received adjuvant or neoadjuvant chemoradiation. We hypothesized that *RECQL* (i) would be mutated in cancer, (ii) would have polymorphisms linked to the 3' UTR A159C and that either or both events would affect function. We also hypothesized that (iii) these changes would be associated with survival in both cohorts of patients.

**Material and methods:** We sequenced *RECQL*'s 15 exons and surrounding sequences in paired blood and tumour DNA of 39 patients. The 3' UTR A159C genotype was determined in blood DNA samples from 176 patients with resectable pancreatic adenocarcinoma treated with adjuvant (53) or neoadjuvant (123) chemoradiation. Survival was calculated using the Kaplan–Meier method, with log rank comparisons between groups. The relative impact of genotype on time to overall survival was performed using the Cox proportional hazards model.

**Results:** Somatic mutations were found in UTRs and intronic regions but not in exonic coding regions of the *RECQL* gene. Two single nucleotide polymorphisms (SNPs), located in introns 2 and 11, were found to be part of the same haplotype block as the *RECQL* A159C SNP and showed a similar association with overall survival. No short-term difference in survival between treatment strategies was found. We identified a subgroup of patients responsive to neoadjuvant therapy in which the 159 A allele conferred strikingly improved long-term survival.

**Discussion:** The *RECQL* 3' UTR A159C SNP is not linked with other functional SNPs within *RECQL* but may function as a site for regulatory molecules. The mechanism of action needs to be clarified further.

## Keywords

*RECQL*, *RECQL1*, polymorphism, resectable pancreatic adenocarcinoma, neoadjuvant and adjuvant therapy

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## Introduction

With an estimated 37 680 new diagnoses and 34 290 deaths in 2008, cancer of the pancreas is the eleventh most common cancer in the United States and fourth in fatalities.<sup>1,2</sup> Most American centres treat resectable pancreatic cancer with resection followed by adjuvant chemoradiation, which extends survival from 12 months with surgery alone to about 20 months with adjuvant chemoradiation, with a 20% 5 year post-operative survival. Conversely, the neoadjuvant approach, with assured administration of chemoradiation to all potential surgical candidates and potentially improved patient tolerance prior to surgery, offers some theoretical advantages over immediate surgery. Pre-operative treatment may decrease tumour size and increase the chances of an R0 (microscopically negative margin) resection. However, about 30% of patients treated with pre-operative chemoradiation will develop progressive disease and will never come to surgery. Clearly, discovery of a means to predict the outcome with the adjuvant or neoadjuvant approach would be extremely useful.

Gemcitabine (GEM) is the standard chemotherapy for pancreatic cancer. GEM interferes with DNA replication and prevents the proofreading enzymes from detecting, excising and repairing the DNA.<sup>3</sup> *In vitro* studies have shown that cells deficient in DNA mismatch repair (MMR) enzymes are more sensitive to GEM/radiation.<sup>4</sup> The RecQ family is a highly conserved group of DNA helicases required for the maintenance of genome stability and integrity. They have an important role in DNA replication, telomere maintenance, DNA damage signalling and DNA repair pathways including mismatch repair, nucleotide excision repair and direct repair.<sup>5,6</sup> The most highly expressed but smallest member of this family in human cells is the RecQ protein-like (DNA helicase Q1-like) (*RECQL* often named RECQ1). *RECQL* catalytic activities include the DNA unwinding of diverse but specific DNA structures, the annealing of complementary single-stranded DNA and DNA branch migration.<sup>7–11</sup> This protein plays an important role in chromosomal stability and genome maintenance.<sup>11</sup> It interacts with and binds to mismatch repair proteins that regulate genetic recombination<sup>12</sup> and participates in the repair of endogenous or exogenously induced DNA damage.<sup>13</sup> *RECQL* is highly expressed in rapidly proliferating cancer cells and transformed cells and provides these cells with a growth advantage<sup>14,15</sup> suggesting that greater copy numbers of *RECQL* may be needed to repair the elevated load of endogenous DNA damage generated during their accelerated cell cycle. Accordingly, *RECQL* acute depletion (silencing by small interference RNA) in cancer cells induces growth retardation, sensitivity to DNA damaging agents, accumulation of DNA damage, chromosomal instability and ultimately results in mitotic catastrophe coupled with mitotic cell death in cancer cells already compromised in their checkpoint.<sup>12,15</sup> Conversely, allelic loss at the *RECQL* locus (chromosome 12p12) is frequent in different tumour types.<sup>11</sup>

Alteration in DNA repair pathways may affect the cytotoxicity of chemotherapy and radiotherapy, more specifically the resistance to gemcitabine-induced DNA replication arrest and the repair of DNA double-strand breaks caused by radiation. The importance of *RECQL* in damage repair is further supported by the observation that a single nucleotide polymorphism (SNP) located in the 3' untranslated region (UTR) (A159C) negatively affects the overall survival and response to gemcitabine-induced radiosensitization of patients diagnosed with pancreatic adenocarcinoma treated with neoadjuvant chemoradiation.<sup>16,17</sup> This SNP exerts its effect in a dominant-inheritance mode.

Genetic changes can be considered either germline or somatic. Germline variation refers to an alteration in DNA sequence inherited from one's parents. Single nucleotide polymorphisms (SNPs) account for more than 90% of germline variation in the human genome, and have been implicated in phenotype, disease predisposition and response to therapy.<sup>18,19</sup> Somatic mutations, to the contrary, are found in diseased tissue (i.e. tumour) only, and are not a part of the inherited genetic complement. By altering protein function, somatic mutations can have a profound impact on tumour development and proliferation (e.g. tumour suppressor genes and oncogenes).

In the present study, the *RECQL* gene was sequenced in patients with pancreatic cancer to discover the presence of additional SNPs or mutations in the coding region that may directly affect the function of the gene. In addition, to evaluate the prognostic value of the 3'UTR A159C SNP in association with the course of treatment, the effect of the SNP on clinical outcome was compared in two cohorts of patients with resectable pancreatic cancer: one treated with resection and adjuvant chemoradiation, and the other treated with neoadjuvant chemoradiation with surgery or additional chemotherapy as indicated by repeat CT scan.

## Material and methods

### Sample collection and processing

Informed consent from patients with resectable pancreatic exocrine adenocarcinoma was obtained under an institutional review board approved protocol at the institutions participating in this study (BCM: H16215 issued 9/22/04; TJU: 06U.76 issued 4/27/06; MDA: ID 98–155 issued 09/09/98). A total of 53 patients with exocrine adenocarcinoma located in the head of the pancreas were treated with pancreaticoduodenectomy (Whipple procedure) and adjuvant chemoradiation at the Elkins Pancreas Center at Baylor College of Medicine (BCM), Houston, and the Thomas Jefferson University Center (TJU) for Pancreatic, Biliary and Related Cancers, Philadelphia (26 and 27 patients, respectively). One hundred twenty-three patients received neoadjuvant chemoradiation at the University of Texas MD Anderson Cancer Center (MDACC).

The blood from the BCM patients was directly collected in PAXgene Blood DNA tubes, and the DNA was isolated with the PAXgene Blood DNA kit (PreAnalytiX; Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The MDACC

blood samples were collected in heparinized vacutainers (BD Biosciences, Franklin Lakes, NJ, USA), and the peripheral mononuclear cells were immediately separated by Ficoll-Histopaque (Amersham Pharmacia Biotech, Piscataway, NJ, USA) density gradient centrifugation; the DNA was then extracted with the FlexiGene DNA kit (Qiagen). At TJU, after surgical resection, genomic DNA was isolated from normal and tumour pancreatic tissue. gDNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen).

Sequencing data were obtained from 39 macroscopically dissected pancreatic adenocarcinoma tissue specimens from operative cases (head, tail and metastasizing tumours) and matching blood from the same patient (BCM). The tissue samples were stored in a protease inhibitor solution (Roche Applied Science, Indianapolis, IN, USA) at  $-80^{\circ}\text{C}$ .<sup>19,20</sup> The DNA was isolated from the preserved tissue using the QIAamp DNA Mini kit (Qiagen) after washing the tissue several times in phosphate-buffered saline (PBS) to remove any trace of the stabilizing solution.

### Gene amplification and sequencing

The exons and their flanking sequences (300 bases) of *RECQL* were identified using the UCSC genome bioinformatics website (<http://genome.ucsc.edu>; accessed 18 June 2009). Primer sets (Table 1) were designed using the HGSC primer design pipeline that links several software in an integrated automatic approach as previously described.<sup>19</sup>

Ten to fifty nanograms (ng) of DNA was whole genome amplified (WGA) (GenomiPhi DNA amplification kit, Amersham Biosciences). PCR was performed on 10 ng of WGA DNA in a final reaction volume of 8  $\mu\text{l}$  in a 384-well plate using the HotStar polymerase (Qiagen). Cycling parameters consisted of 40 cycles of a denaturation step at  $95^{\circ}\text{C}$  for 45 s followed by an annealing step at  $60^{\circ}\text{C}$  for 45 s, and an extension step at  $72^{\circ}\text{C}$  for 45 s. The cycling process was preceded by a denaturation period at  $95^{\circ}\text{C}$  for 15 min and followed by a final extension period of 7 min at  $72^{\circ}\text{C}$ . Unconsumed dNTPs were hydrolyzed and remaining primers were degraded using a cocktail of Shrimp Alkaline Phosphatase and

**Table 1** Primer sets and probes used for sequencing, validation and genotyping

Sanger sequencing primer sets			
Exon	Forward primer	Forward primer	
1	TAACTTTCCGGTTTCTCCTCCG	TTCCTATTGGCGAAACCTGCTT	
1	TGGAGGAAACGCCACTGAGATA	AGCTTTGAAGGGTCAAGGGTGT	
2	CAACAGAAATAGAACAGAAGGAAGAAGA	TGGTCTTATTGAAAGGTCACTGC	
3	CTGCAAGTTTCCATTCCACTG	CTGTAATTGATATGGCGGGCAA	
4	TGACAAAGCACTTCTTCAACTCAAA	TTTGCTTTGCTTAGCTAGTGAGT	
5	GCAGGTAAAGCTCCTATTTCACTG	GCATCTGATTCTGAGGGTGGTG	
6	AGCAGAGATTTCCATCATGCCA	TGCTCCTAGAAGAGCCAAAGGC	
7	CCCTCTGCGTAATTCTTCACAAA	CCTGCCCATCAAAGAGGCTAAC	
8	TCTCTGTCTCCAAAGTTGGTTTG	TCTGGGAGGAGGAACAGATGAG	
9	TCAATTCCTACCAATGCAA	CAATGTCCTGAATGTGTGTGTTGA	
10	CCATATGCAAGTAAGTGTCAAACCTGC	TGCTTACCATGCCAATTTGGAG	
11	GATGTGCTGGTTCCTACCCTCC	GGGTACCCATTACAAATTAACCTCCAC	
12	CACTAGGCATTATGACTGTATTAGCC	ATCCAGTGAAACCCATAGTACACTTC	
13	AAACCGTTTATTCTGTGCAATTT	AGGGTGGCTCACATTGATAACC	
14	CAATGTGTTAAGAATTACTGCGCATA	GAAAAGCATCCCATAGGCTTT	
15	AAACTTAAGACGATTGTATGAACCTATTCTC	TTTGTAGGCTGAATCGTCTCAAAC	
15	AAACTTAAGACGATTGTATGAACCTATTCTC	GAAAAGAAAAATCGATGATGCCT	
15	CACTCAGTGAACCTCTGTCACT	TTTGAGAATAAGTTCATACAATCGTC	
Biotage primer set			
SNP	Forward primer	Reverse primer	Sequencing primer
Intron 2, IVS2 – 17	TTTCCGTAAGTTCTTGAATTTG	TCAATCTGGAAACCTAAAGTTGTA	CTATGGGAGGCAGCG
intron 11, IVS11 + 30	GGGCATCTTTTCTTGAACCTAAGG	GCAGAAGCTTTATGAGATGGTATC	CTTTCATATTTGCTTTAATT
3' UTR, A159C	AATAATGGCATATACATGCATAAA	ACAGGAGCTAAGAAAAAGAAAAATC	TCGATGATGCCTGATA
TaqMan primer and probe set			
SNP	Forward primer	Reverse primer	Probe
3' UTR, A159C	CAATCTGGTTCTAAGAATACAGGAGCTA	GGCATATACATGCATAAACCATCTTT	AGTAACAGTCATATCAGG AGTAACATTATATCAGGC

Exonuclease I (ExoSAP-IT, USB). The purified PCR products were diluted and sequenced using BigDye Terminator version 3.1 Cycle Sequencing kit on Applied Biosystems (Foster City, CA, USA) 3730XL DNA Sequencers. The sequences were analysed with SNP Detector version 3 (created by Jinhui Zhang *et al.*<sup>21</sup> at the NCI) using the corresponding sequence deposited in GenBank as reference. Base disparities from the reference sequence identified by the SNP Detector software were manually verified in Sequencher version 4.6 (Gene Codes Corp). Somatic variants were identified by comparing the sequences from each patient's matched blood and tumour samples.

### Validation and genotyping

We validated the selected candidate variants by Biotage pyrosequencing. Briefly, Biotage assay design software was used to select new PCR primers at each locus of interest, avoiding all known SNPs and project-specific variants (Table 1). One of each primer pair was tailed with a 23 base extension corresponding to the sequence of a biotinylated universal primer. Primers successfully tested for amplification product on human cell line DNA were combined with the universal primer and used to amplify patient DNA samples. Each amplicon was reduced to single strand by denaturation and binding to streptavidin beads. Sequence-specific extension primers were annealed within four bases of the target variant and extended using the standard Biotage pyrosequencing protocol and reagents. The resulting 8 to 15 bases of sequence were analysed for quality based on pyrogram peak geometry and genotype using Biotage software algorithms.

The 3'UTR A159C SNP was also genotyped with the *TaqMan* SNP Genotyping assay (Applied Biosystems) (Table 1). The reactions were prepared using 10 ng of gDNA, *TaqMan* universal master mix and SNP genotyping assay mix in a final volume of 5 µl. The PCR was done using the ABI Prism 7900 HT sequence-detector.

### Statistical methods

Estimations of survival were performed via the Kaplan–Meier method, with comparisons in survival between groups made pairwise by log-rank tests. Differences in distribution of variables between groups were calculated using Pearson's  $\chi^2$ -test. Potential confounders were identified by univariate analysis; individual variable's relative effect on survival was estimated via a Cox

regression model. All statistical calculations were performed with SPSS version 12.0.1 (SPSS Inc., Chicago, Illinois, USA).

## Results

### Sequencing results: somatic events (specifically found in tumour DNA only)

We sequenced the 15 exons and surrounding intronic regions of the *RECQL* gene in 39 matched blood and tumour samples of patients diagnosed with pancreatic adenocarcinoma resected before chemoradiation. A total of 26 events were identified (Table 2). Out of these events, two consisted of a somatic base shift mutation located in the 5'UTR, and intron 1 in two patient tumours (Tables 2,3). Six other somatic events located in five different introns as well as the 3'UTR A159C consisted of loss of heterozygosity (LOH) at the site of known polymorphisms. Most of these somatic events were found in two patients (Tables 2,3).

### Sequencing results: germline polymorphisms (found in blood as well as tumour DNA)

The remaining events consisted of 24 polymorphisms, of which 15 were known to be present in the general population, and had an assigned ID number (<http://genewindow.nci.nih.gov>; accessed 18 June 2009). Fourteen SNPs (nine known) were located in the intronic region outside the splicing site, six SNPs (three known) in the UTRs of the gene, one known SNP 115 bases upstream of exon 1, and three SNPs in the coding region of exon 13 (unknown missense SNP), exons 14, and 15 (both silent known SNPs) (Table 3). The impact of the missense mutation on *recql* function was suggested to be benign based on a PolyPhen analysis (<http://genetics.bwh.harvard.edu/pph/>; accessed 18 June 2009).

Nine SNPs were part of three haplotype blocks (Table 3): haplotype 1: IVS2 – 17, IVS11 30, Ex15 + 159 (3' UTR A159C); haplotype 2: IVS7 – 69, IVS8 – 33, IVS10 + 82, and IVS11 + 103; and haplotype 3: Ex14 + 64 and Ex15 + 102. First we genotyped eight of the SNPs showing greater heterozygosity in DNA extracted from the blood of 77 patients receiving neoadjuvant therapy. Only three SNPs had any correlation with overall survival: they were part of the first haplotype block described above. The total number of genotyped patients was then increased to 123 and the overall survival curves by genotype were established for each SNP. From the three SNPs, the 3'UTR A159C was the most statistically significant (Fig. 1).

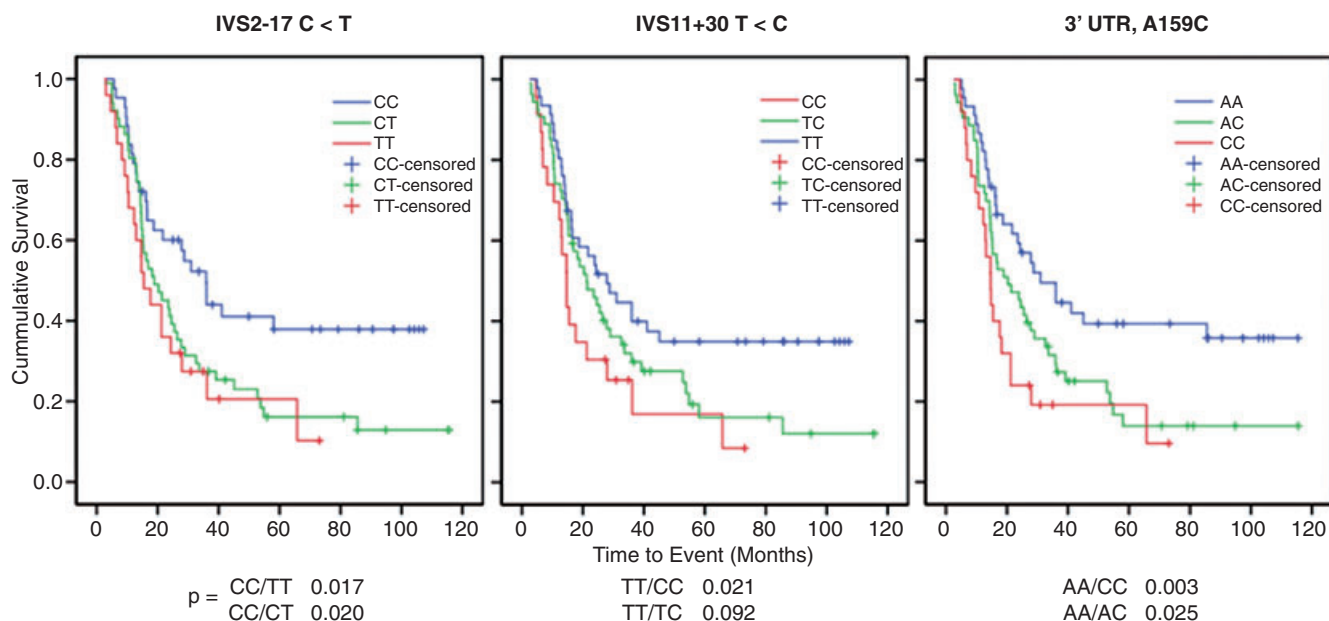
**Table 2** *RECQL* germline and somatic events identified in blood and matching tumour of patients with pancreatic exocrine adenocarcinoma

Gene region	Total event	Germline SNP	Somatic	
			LOH	Base shift
Promoter	1	1		
Intron	15	14	5	1
Exon: UTR	7	6	1	1
Coding: synonymous	2	2		
Coding: nonsynonymous	1	1		

Table 3 Somatic and germline events detected in the matching blood and tumour samples of patients treated with adjuvant therapy

Chrom location (hg 18)	Gene location, function	Official nomenclature	Base shift	SNP ID	Total patient number			Genotype		
					Germline		Somatic			
					Homo major	Hetero	Homo minor	Amount	Type	Patient ID
Somatic events: mutations and LOH										
21545758	5' UTR, CDS -433 bases	Ex1 + 39	G > A		39	38		1	Base shift	58
21545258	Intron 1	IVS1 + 113	G > A		38	37		1	Base shift	2440
21543908	Intron 1	IVS1 - 92	C > T	rs12423412	38	22	13	2	LOH	6030
21535934	Intron 2	IVS2 - 17	C > T	rs10841834	39	10	15	11	LOH	57, 58, 6030
21520058	Intron 8	IVS8 - 33	A > G	rs3752648	39	13	18	7	LOH	58
21519587	Intron 10	IVS10 + 82	G > A	rs10841831	38	13	16	7	LOH	58,4520
21519012	Intron 11	IVS11 + 30	T > C	rs2159943	39	9	20	9	LOH	6030
21514389	3' UTR, CDS +6 bases	Ex15 + 159	A > C	rs13035	39	10	18	10	LOH	6030
Germline polymorphisms in exons and their immediate surrounding regions:										
21545759	5' UTR, CDS -434 bases	Ex1 + 38	G > A		39	37	1	1		
21545736	5' UTR, CDS -411 bases	Ex1 + 61	A > C	rs1061626	39	29	10			
21545674	5' UTR, CDS -349 bases	Ex1 + 123	T > C	rs1061627	39	25	14	1		
21545532	5' UTR, CDS -207 bases	Ex1 + 265	C > T		39	36	3			
21515831	Ile489Val, missense	Ex13 + 18	A > G		39	38	1			
21515236	Asn577Asn, silent	Ex14 + 64	T > C	rs6500	39	34	5			
21514446	Gln633Gln, silent	Ex15 + 102	A > G	rs17849408	39	34	5			
21514194	3' UTR, CDS +210	Ex15 + 360	A > G		39	38	1			
21545911	Promoter, Exon 1 -115 bases	-2139	T > G	rs1860947	39	32	6	1		
21535933	Intron 2	IVS2 - 16	G > C		39	38	1			
21534665	Intron 3	IVS3 - 86	G > A	rs4987216	39	36	3			
21534630	Intron 3	IVS3 - 51	C > T		39	38	1			
21521262	Intron 7	IVS7 - 69	- > G	rs5796903	39	13	19	7		
21518939	Intron 11	IVS11 + 103	G > C	rs35159698	38	13	18	7		
21515584	Intron 13	IVS13 + 45	T > -		37	29		8		
21515576	Intron 13	IVS13 + 53	T > A	rs11046076	37	8	9	20		
21515571	Intron 13	IVS13 + 58	A > -		37	26	11			
21515155	Intron 14	IVS14 + 15	TAA > -		39	38	1			

CDS, coding sequence; UTR, untranslated region; homo, homozygote; hetero, heterozygote.



**Figure 1** Comparison of the overall survival by genotype of three single nucleotide polymorphisms (SNPs) from the same haplotype. The genotypes CC, TT and AA at IVS32 – 17, IVS11 + 30, and at the base 159 of exon 15 in the 3' untranslated region (UTR) confer a similar overall survival advantage to the 123 patients that received neoadjuvant therapy. The 3'UTR A159C has a greater statistical significance.

### The 3'UTR A159C polymorphism: correlation between survival and course of treatment

We compared the influence of the 3'UTR A159C SNP on overall survival between patients treated with neoadjuvant chemoradiation and adjuvant therapy. Characteristics of the two cohorts of patients are detailed in Table 4. The two groups were similar in age, race, pre-operative CA 19-9 and TNM stage. There was no difference in overall survival between the two groups (Fig. 2). Although there were differences in gender, the number who underwent resection, tumour size and nodal status, only negative nodal involvement offered a survival advantage using Cox regression (HR 0.420, CI 0.233–0.760,  $P = 0.004$ ).

We determined the germline genotype from the blood DNA of 123 patients receiving neoadjuvant therapy and 53 patients receiving adjuvant therapy. No significant difference was observed in the genotype distribution among the two groups: the frequency of the A allele was 56.6% vs. 58.1%, respectively (Table 5). No statistically significant difference in short-term survival was observed when comparing patients treated by the neoadjuvant vs. adjuvant approach within each respective genotype (Table 6). For example, in comparing neoadjuvant and adjuvant therapy in patients with the AA genotype, survival was 82% vs. 75% in the 1st year and 59% vs. 45% for the 2nd year. This represented no significant difference between the two treatment cohorts.

When comparing the effect of genotype within treatment groups, patients with the AA genotype receiving neoadjuvant chemoradiation were observed to have improved long-term survival compared with their AC and CC counterparts (Fig. 1, Table 6). Five-year survival was 39% for AA patients, compared with 14% for genotype AC, ( $P = 0.025$ ) and 19% for genotype CC ( $P = 0.003$ ). The AA genotype was an independent predictor of improved survival by Cox regression (HR 2.51, CI 1.321–4.756,  $P = 0.005$ ). No long-term survival data were available for the patients receiving adjuvant chemoradiation. However, no statistically significant difference in short-term survival by genotype was seen after 30 months.

We further analysed the population that received neoadjuvant therapy by dividing these patients into two groups based on resectability after chemoradiation. A survival benefit was again observed with the AA genotype in those undergoing surgical resection after chemoradiation, with survival of 97%, 80%, and 55% at 1, 2 and 5 years, respectively vs. 89%, 62%, and 19% for AC patients ( $P = 0.010$ ), and 93%, 36%, and 29% for CC patients ( $P = 0.003$ ) (Fig. 3, Table 7). The AA genotype was again a predictor of survival (HR 2.92, CI 1.531–5.564,  $P = 0.001$ ). However, none of the genotypes conferred a survival benefit in patients that could not undergo resection as a result of progressive disease during neoadjuvant chemoradiation (Fig. 3). These patients had a 2-year survival of 10% for all genotypes. The difference in survival was highly emphasized when the outcomes of each genotype were compared (Fig. 4).



**Table 4** Patient demographics

		Neoadjuvant 123 patients	Adjuvant 53 patients
Gender <sup>a</sup>	Female	38.2%	58.5%
	Male	61.8%	41.5%
Age group	<50	12.2%	3.8%
	51–60	22.8%	15.4%
	61–70	38.2%	38.5%
	>71	26.8%	42.3%
Race	Caucasian	88.6%	81.1%
	Hispanic	4.9%	3.8%
	Black	4.9%	9.4%
	Asian	1.6%	5.7%
CA 19-9 level	<47	26%	26.8%
	48–499	48%	39%
	500–999	11.4%	12.2%
	>999	14.6%	22.0%
Surgical resection <sup>a</sup>	Resected	65.9%	100%
	Non-resectable	34.1%	0%
Tumour size <sup>a</sup>	<2 cm	42.2%	14.0%
	>2 cm	57.6%	86.0%
TNM stage	IA	7.8%	1.9%
	IB	2.6%	3.8%
	IIA	31.2%	21.2%
	IIB	58.40%	73.10%

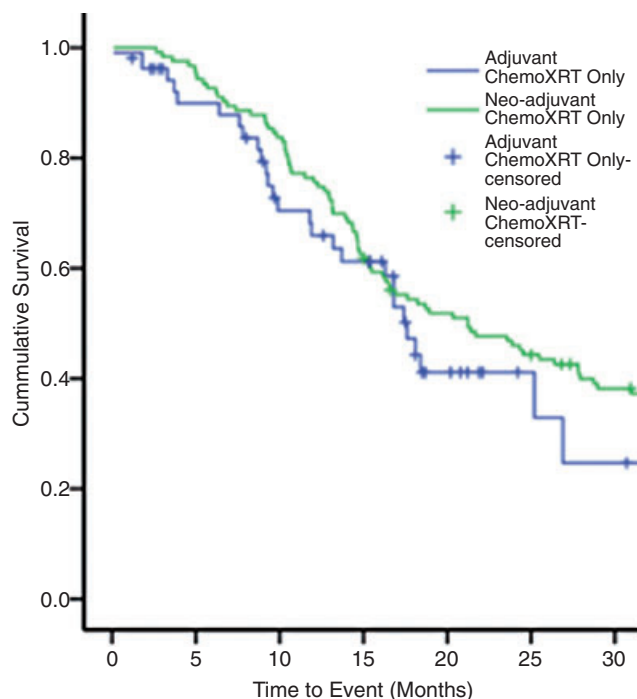
<sup>a</sup>Significant difference between the two groups ( $P < 0.05$ ).

## Discussion

Although this study provided a more thorough sequencing of the *RECQL* gene in the pancreatic cancer patient population, it failed to identify mutations in the coding region of the gene that would impair its functionality. Only a few patients harboured an LOH at the gene locus as previously observed in other cancer types.<sup>11</sup>

In this study, two SNPs in introns 2 and 11 of the *RECQL* gene were identified as being in linkage disequilibrium with 3' UTR A159C and had a similar effect on clinical outcome. Therefore, the mechanism of the association is unlikely to be a SNP resulting in amino acid substitution and subsequent structural and functional defects in the translated *RECQL* protein. The *RECQL* polymorphism may represent a binding site for regulatory molecules that affect gene expression. Also, the polymorphism may impact GEM's mechanism of action. Both of these hypotheses will require further investigation.

The 3'UTR A159C SNP is present at different frequency among different ethnicities as reported by the HapMap project and other studies ([http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?rs=13035](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=13035); 18 June 2009). The frequency of the A allele is about 55% in the Asiatic population, 57% in the Caucasian and 90% in the Yoruban population. The frequency of the AA genotype is as low



**Figure 2** Cumulative overall survival of patients treated with adjuvant and neoadjuvant therapy. No difference was noticed between the two groups.

as 16% in some Asiatic ethnicities, about 35% in the Caucasian population, and over 80% in the Yoruban population. We observed a similar genotype distribution among our pancreatic cancer patients.

The present study adds further information to the observation that the well known and validated *RECQL* polymorphism, 3'UTR A159C, is associated with overall survival of patients with resectable pancreatic adenocarcinoma. No significant difference in short-term survival was observed by genotype between the patients treated with neoadjuvant and adjuvant therapy. A key weakness of this study is the small sample size and limited follow-up, particularly in the group treated with adjuvant chemoradiation. Long-term follow-up of these patients is necessary to see if trends in survival similar to that seen in the neoadjuvant group develop.

Similar to previously reported series, 34% of patients treated neoadjuvantly progressed to unresectable disease. The subset of unresectable neoadjuvant patients in our population experienced extremely poor clinical results, with most dying within a year of diagnosis. Clearly, the genotype and other factors that determine response to treatment and overall survival are multiple and complex. Factors, including mutations in genes impacting tumour behaviour or regulatory molecules, may be involved in worsening patient survival in this subset. Chen *et al.* have also reported polymorphisms in cell-cycle genes that had an impact on overall survival in patients with resectable adenocarcinoma treated with neoadjuvant chemotherapy.<sup>22</sup> It is also possible that this subgroup

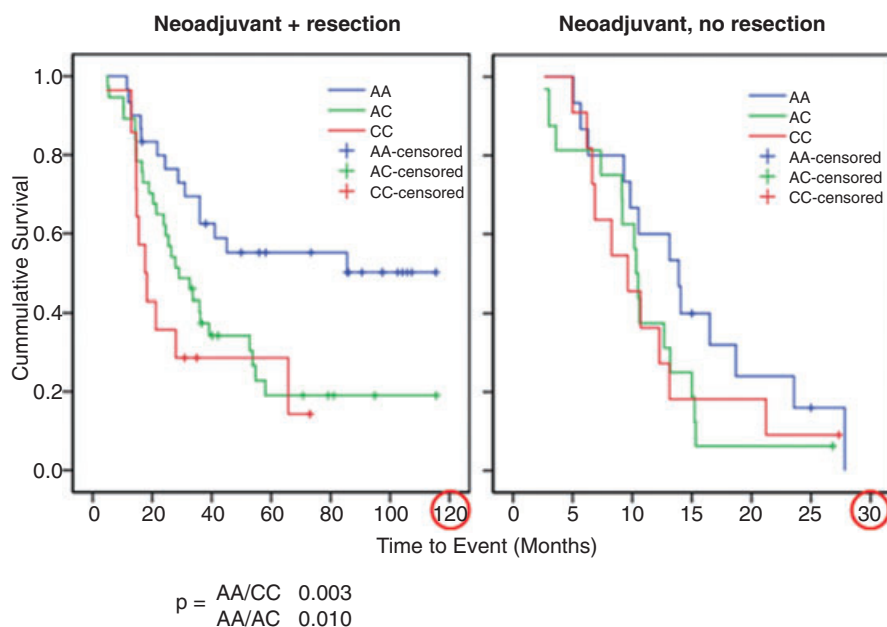
**Table 5** RECQL A159C genotype distribution among the groups studied

	Total	AA	AC	CC	% A allele
Adjuvant therapy					
All patients	53	34.0%	45.3%	20.8%	56.6%
Neoadjuvant therapy					
All patients	123	36.6%	43.1%	20.3%	58.1%
Resected	81	37.0%	45.7%	17.3%	59.9%
Unresected	42	35.7%	38.1%	26.2%	54.8%

**Table 6** Survival comparison between the patients treated with neoadjuvant and adjuvant therapy

Genotype	AA		AC		CC	
	Neoadjuvant	Adjuvant	Neoadjuvant	Adjuvant	Neoadjuvant	Adjuvant
1 year	82%	75%	74%	70%	68%	45%
2 years	59%	45%	45%	52%	24%	34%
5 years	39%	na	14%	na	19%	na

na, not available yet.



**Figure 3** Comparison of the overall survival by genotype between two subgroups of patients receiving neoadjuvant therapy based on their resectability after chemoradiation. Patients with the AA genotype receiving the entire course of treatment (chemoradiation and surgery) had an impressive overall survival of 55% over 5 years. Conversely, no difference in overall survival was associated with any genotype for patients unresectable after neoadjuvant therapy.

of patients had already developed micrometastases that were undetected at staging prior to the initiation of neoadjuvant therapy.

Within the specific subgroup of patients responsive to neoadjuvant therapy who proceeded to resection, the AA genotype is associated with a relatively promising long-term survival, making it a potentially good prognostic marker for this group. However, the AA genotype was in equal prevalence in patients who proceeded to resection as well as those who failed neoadjuvant therapy. It does not appear, then, that the 3'UTR A159C genotype can predict which patients will respond to neoadjuvant

chemoradiation. Thus, the clinical utility of this marker is limited until a means of prospectively identifying this responsive subgroup is established.

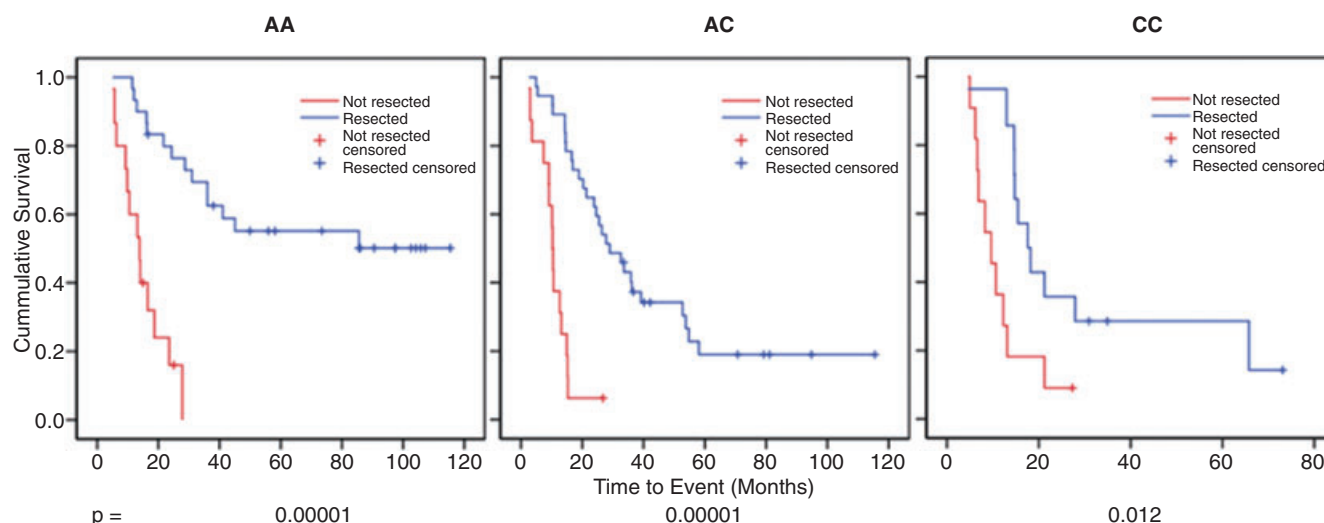
## Conclusion

Recent advances in rapid DNA sequencing technology are decreasing the cost and improving the efficiency of genetic testing such that selection of therapy based on genetic analyses of individual patients may soon become standard practice. This personalized genomic approach to cancer therapy may improve



**Table 7** Survival comparison between the original group of patients treated with neoadjuvant therapy and two subgroups: patients proceeding to resection after chemoradiation and patients for which resection was not feasible because of progression

Genotype	AA			AC			CC		
Group	All patients	Resected	Unresected	All patients	Resected	Unresected	All patients	Resected	Unresected
1 year	82%	97%	60%	74%	89%	37.5%	68%	93%	36.4%
2 years	59%	80%	16%	45%	62%	6.3%	24%	36%	9.1%
5 years	39%	55%		14%	19%		19%	29%	
Mean (month)	56.5	73.8	15.0	35.2	44.6	10.9	24.0	31.4	11.6



**Figure 4** Comparison of the overall survival by genotype between patients receiving the full course of neoadjuvant therapy with resection and patients unresectable after chemoradiation. For any genotype, the patients unable to receive a full course of treatment had a significantly worsened survival.

results by selection of more beneficial treatments for each patient and by avoiding the application of treatments that are ineffective and toxic.<sup>23</sup> In an era where the cost-benefit ratio of treatments will be increasingly scrutinized, selection of patients with pancreatic cancer, who have a universally fatal disease, that would be likely to benefit from otherwise highly toxic and expensive treatment modalities may become critical. The present study retrospectively identified a distinct subset of pancreatic cancer patients in whom a specific genotype was associated with a nearly threefold increase in survival over that which is typically reported in the literature. Current and future studies, including whole-genome analysis of this population, may yield clinically relevant data that can influence prospective medical decision making and choice of therapy.

#### Conflicts disclosure

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